Origin of ultradian pulsatility in the hypothalamic-pituitary-adrenal axis

Jamie J. Walker[1,*], John R. Terry[1] and Stafford L. Lightman[2]

[1]*Bristol Centre for Applied Nonlinear Mathematics, Department of Engineering Mathematics, University of Bristol, Bristol, United Kingdom*

[2]*Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, University of Bristol, Bristol, United Kingdom*

[*]*E-mail: Jamie.Walker@bristol.ac.uk*

Summary

The hypothalamic-pituitary-adrenal (HPA) axis is a neuroendocrine system that regulates the circulating levels of vital glucocorticoid hormones. The activity of the HPA axis is characterized not only by a classic circadian rhythm but also by an ultradian pattern of discrete pulsatile release of glucocorticoids. A number of psychiatric and metabolic diseases are associated with changes in glucocorticoid pulsatility, and it is now clear that glucocorticoid responsive genes respond to these rapid fluctuations in a biologically meaningful way. Theoretical modeling has enabled us to identify and explore potential mechanisms underlying the ultradian activity in this axis, which to date have not been successfully identified. We demonstrate that the combination of delay and feedforward and feedback loops in the pituitary-adrenal system is sufficient to give rise to ultradian pulsatility in the absence of an external ultradian source from a supra-pituitary site. Moreover, our model enables us to predict the different patterns of glucocorticoid release mediated by changes in hypophysial-portal corticotrophin-releasing hormone (CRH) levels, with

results that parallel our experimental in vivo data. Since the vast majority of hormones are secreted in ultradian patterns, and most endocrine systems involve excitatory/inhibitory pathways and delays, our theoretical approach could provide a basis for understanding the origin and regulation of the ultradian rhythmicity seen in many other endocrine systems.

Keywords: neuroendocrine regulation; glucocorticoid hormones; ultradian pulsatility

Introduction

Frequency of coding of intercellular signals is a well accepted mode of communication between neurons. More than this however, it is actually a common mechanism of communication across a broad range of both inter- and even intra-cellular systems (Goldbeter 1996). Even an organism as primitive as the slime mould (*Dictyostelium discoideum*) only aggregates in response to external pulses of cyclic AMP delivered with a periodicity of five minutes and not to constant stimuli or frequencies greater than every two minutes (Darmon *et al.* 1975).

Within mammalian systems, frequency encoding is mediated by circulating hormones as major signalling molecules, and many of these signal through mechanisms of ultradian rhythmicity. Thus pulsatile gonadotropin-releasing hormone (GnRH) release is essential for maintenance of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion (Belchetz *et al.* 1978), and modulation of GnRH pulse frequency can produce differential LH and FSH secretion (Wildt *et al.* 1981) via regulation of LH beta and FSH beta mRNA expression (Papavasiliou *et al.* 1986). Similarly, differences in episodic release of growth hormone (GH) elicit significant

differences in gene expression (Waxman *et al.* 1995).

Another system that is characterised by an ultradian rhythm is the hypothalamic-pituitary-adrenal (HPA) axis (figure 1). This stress responsive neuroendocrine system is extremely well adapted to respond to homeostatic challenge. The HPA axis governs the circulating levels of vital glucocorticoid hormones (CORT), which in turn have major regulatory effects on the cardiovascular, metabolic, cognitive and immunological state of the animal (Chrousos 1995; de Kloet *et al.* 2005; McEwen 2007). The central regulator of this axis - the paraventricular nucleus (PVN) of the hypothalamus - is a major relay for afferent information from limbic areas of the central nervous system that can detect cognitive or emotional stressors, and also from brain stem structures that detect more physical stressors such as inflammation or hypotension (Ulrich-Lai & Herman 2009). The PVN also receives a major input from the hypothalamic suprachiasmatic nucleus (SCN) that coordinates the body's circadian rhythms (Reppert & Weaver 2002). The PVN in turn projects to the median eminence of the hypothalamus from where it releases corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) into the hypothalamic-pituitary portal circulation. The CRH and AVP pass along this vascular route to access their receptors on corticotroph cells in the anterior pituitary. These cells in turn are activated by occupation of their CRH and AVP receptors to release corticotrophin (ACTH) into the general circulation through which it accesses the glucocorticoid secreting cells in the cortex of the adrenal gland. It is these cells that synthesize and release the final product of HPA activation - the glucocorticoid hormones (CORT). The final link in this circuit is that physiological levels of glucocorticoid hormones themselves feedback in a negative manner on the pituitary gland to inhibit further release of ACTH (Jones *et al.* 1977; Dallman *et al.* 1987).

The HPA axis has a unique pattern of activity. Levels are low during the periods of sleep inactivity and increase in anticipation of waking, peaking in the morning in man (Weitzman *et al.* 1971) and evening in the rodent (Dallman *et al.* 1978), with the resultant classic circadian rhythm. This rhythm however is not made up of a simple smooth change in hormone levels over the 24 hours. The circadian changes of glucocorticoids are a result of changes in activity of an underlying ultradian rhythm (Veldhuis *et al.* 1989; Jasper & Engeland 1991; Windle *et al.* 1998; Spiga *et al.* 2007). Glucocorticoids are actually released from the adrenal gland in discrete pulses which result in rapidly changing levels of hormone, both in the blood and within the tissues (figure 2). It is in fact the changes in pulse amplitude, and to a lesser extent frequency, that make up the circadian rhythm (Lui *et al.* 1987; Iranmanesh *et al.* 1989; Veldhuis *et al.* 1989, 1990; Windle *et al.* 1998) and the changes of HPA activity that occur in response to altered physiological and pathological conditions. This pulsatility of glucocorticoid secretion is also an important factor in determining the responsivity of the HPA axis to stress (Windle *et al.* 1998; Lightman *et al.* 2008) and the transcriptional responses of glucocorticoid responsive genes (Stavreva *et al.* 2009).

Although we have good evidence that the SCN determines the circadian activity of the HPA axis by modulating the inhibitory gain to the PVN, we have no idea of the mechanism responsible for the regulation of ultradian activity. Many people have just presumed there must be some sort of hypothalamic pulse generator; however there is no good evidence for its existence. The only supportive data comes from studies with cultured explants of the macaque hypothalamus (Mershon *et al.* 1992) and rat median eminence (Ixart *et al.* 1991) that show episodic release of CRH. The relevance of this is unclear, particularly in the absence of cyclic feedback of inhibitory signals from circulating glucocorticoids that are now known to have rapid inhibitory effects on

HPA activity. Indeed there is good evidence for the lack of importance of a pulsatile CRH signal from the work of Gerald Lincoln (personal communication), using a model of hypothalamic disconnection from the pituitary in the conscious sheep, which still maintains pulsatile cortisol secretion.

Understanding the mechanisms underlying ultradian HPA activity is very important. It is becoming increasingly clear that glucocorticoid responsive genes respond to these rapid fluctuations in a biologically meaningful way (Stavreva *et al.* 2009) and that a number of psychiatric and metabolic diseases are associated with changes in cortisol pulsatility (Young *et al.* 2004, 2007). Motivated by recent accounts of feedforward and feedback loops supporting robust oscillations in a number of biological contexts (Stricker *et al.* 2008; Tsai *et al.* 2008; Tigges *et al.* 2009), we hypothesized that the pituitary-adrenal system (which contains a positive delayed feedforward connection between ACTH and CORT (Papaikonomou 1977), as well as negative nonlinear feedback of CORT on ACTH mediated by the glucocorticoid receptor (GR) (Drouin *et al.* 1992)) could support ultradian oscillations in the absence of a hypothalamic pulse generator. To address this hypothesis we considered a deterministic theoretical model characterizing the principal interactions between the anterior pituitary and the adrenal cortex (see figure 1, and also figure 7 in the electronic supplementary material). We employed a powerful mathematical technique called numerical continuation (Kuznetsov 1995; Engelborghs *et al.* 2001; Krauskopf *et al.* 2007) - enabling us to systematically characterize how the behaviour of the system depends on the parameters of the system (see the electronic supplementary material for more details) - to explain the mechanisms giving rise to natural oscillatory rhythms in the HPA axis.

Results and Discussion

The aim of model development for the HPA axis was to elucidate whether - using biologically motivated approximations of each of the main compartments of the axis - the system could support ultradian glucocorticoid fluctuations in a similar manner to those observed experimentally, and to explore mechanisms by which these could occur. For this purpose, we adapted a recently proposed model (Gupta *et al.* 2007) using ordinary differential equations (ODEs) which provided a compromise between analytical tractability and biological plausibility. This approach allowed for the integration of experimentally determined parameter values (where known), whilst permitting a theoretical analysis using a simplified model with the potential for refinement using experimental data. Specifically, the model uses linear mass action kinetics to describe the dynamic levels of ACTH, GR and CORT, and incorporates a delay term to account for the well known delay in the CORT response to ACTH which results from the lack of releasable pools of CORT and the need to synthesize the hormone for release (see the electronic supplementary material for more details).

Using numerical simulations and continuation methods (see the electronic supplementary material for more details) we determined a range of values in both CRH drive and delay for which ultradian activity was observed in ACTH and CORT (figure 3*a*,*c*). It is important to stress that these ultradian pulses are an intrinsic property of the pituitary-adrenal system, since they occur in response to a constant level of CRH drive. Furthermore, the ultradian period of the pulses (figure 4 and the colour-bar in figure 3*a*) is consistent with previous experimental studies, which have reported an interpulse interval range between 47.2 and 54.6 minutes (Windle *et al.* 1998).

Interestingly, our simulations demonstrated that only intermediate values of the CRH drive resulted in ultradian pulses, whilst high or low CRH drive resulted in a constant response in ACTH and CORT levels (figure 3*a,b,d*).

Experimental data demonstrates significant changes in the amplitude of ultradian activity over the course of a twenty-four hour period (figure 5*b*). Theoretically, we considered the effect of circadian modulation of the PVN by the SCN by driving the pituitary-adrenal system with a circadian (period of 24 hours) CRH input. Our numerical results parallel experimental observations (Windle *et al.* 1998), whereby the amplitude increases markedly (and the frequency increases slightly) during the high drive CRH input (figure 5*a,d*). Perhaps most significantly, when we included stochastic effects as well as a circadian modulation of the CRH drive, we observed so-called noise-induced coherent oscillations (Wiesenfeld & Moss 1995; Gammaitoni *et al.* 1998) (NICO), for values of the CRH drive close to (but below) the transition curve (beyond which ultradian pulses were observed in the noise-free scenario). These NICO closely resembled the experimental data (figure 5*b,c*) providing evidence for the hypothesis that feedforward and feedback interactions within the pituitary-adrenal system are the foundation of ultradian activity observed experimentally (see also figure 8 in the electronic supplementary material for more examples).

We also considered the effect of ultradian CRH pulses on the response of the pituitary-adrenal system. Experimental work has reported a pulsatile pattern of CRH release from the median eminence of the hypothalamus in the rat, with a mean frequency of three pulses per hour (Ixart *et al.* 1991). Our numerical work shows that the pituitary-adrenal system responds to ultradian CRH pulses differently depending on the precise level of these pulses. If their level lies within the

region of constant response (figure 5*a*), then the pituitary-adrenal system responds with pulses of CORT at the same frequency as the driving CRH pulses (figure 5*f*). Alternatively, if the level of the CRH pulses lies within the region of pulsatile response (figure 5*a*), then the pituitary-adrenal system responds with pulses of CORT at a frequency governed by the intrinsic properties of the pituitary-adrenal system (figure 5*e*).

Finally, we illustrate how this theoretical approach to understanding the ultradian glucocorticoid rhythm can aid the planning of both experimental and clinical trials. One very important area of clinical medicine that has been linked to both over- and under-activity of the HPA axis is the mood disorders. Depression, in particular, has been consistently associated with significant elevations of HPA activity (Holsboer 2001; Pariante 2003), and many studies have shown that this increased activity is associated with a diminution of sensitivity to the negative feedback by endogenous glucocorticoids. This has been demonstrated by data showing a blunting of endogenous glucocorticoid inhibition following administration of the synthetic glucocorticoid dexamethasone, or an inhibition of the ACTH response in the dexamethasone-CRH test (Nemeroff 1996; Holsboer 2000; Pariante & Miller 2001; Pariante 2004). Furthermore, patients suffering from major depression have been found to have reduced levels of GR mRNA in the hippocampus (Webster *et al.* 2002), and glucocorticoid secretion patterns of transgenic mice with reduced GR resemble those patterns seen in subjects with major depression (Pepin *et al.* 1992). Thus the use of GR antagonists (or indeed agonists) clearly has great potential as a therapeutic strategy in treating patients with mood disorders linked to HPA axis dysfunction.

The model we employ here is the first to incorporate the dynamics of the glucocorticoid receptor in the anterior pituitary (Gupta *et al.* 2007), and therefore provides an ideal platform to investi-

gate the effects that GR antagonists/agonists have on the dynamics of endogenous glucocorticoid secretion. Model results demonstrate that infusion of a GR antagonist (such as Org 34850) increases the amplitude of the ultradian glucocorticoid rhythm during the peak of the circadian CRH drive (figure 6*b*). Furthermore there is a minor increase in ultradian frequency under the influence of a GR antagonist. These theoretical observations are consistent with experimental studies on the rat (Spiga *et al.* 2007), where following five days of treatment with the GR antagonist Org 34850, mean corticosterone levels were elevated over the 24hr cycle (figure 6*a*). Furthermore, this general elevation was the result of an underlying increase in both amplitude and frequency of the ultradian pulses. In the same study, analysis of the corticosterone rhythm revealed that Org 34850 had its greatest effect during the peak of the circadian rhythm.

Biological systems utilise rhythmic activity in many time domains, from rapid electrical oscillations in the central nervous system to daily, monthly or even yearly hormone rhythms. The great majority of hormones are also secreted in ultradian patterns which are important for the maintenance of tissue responsiveness and the avoidance of receptor down regulation. The mechanisms underlying these rhythms have been very unclear and in this paper we have been able to show that relatively simple feedforward and feedback interactions between the pituitary and adrenal cortex are sufficient to account for the glucocorticoid rhythms we observe experimentally. More generally, systems with delayed feedforward and feedback pathways typically give rise to oscillations, suggesting that our theoretical approach could provide a basis for understanding the origin and regulation of the ultradian rhythmicity seen in many hormonal systems.

Acknowledgments

The authors acknowledge financial support from the EPSRC via grant EP/E032249/1, and also financial support from The Wellcome Trust via grant 074112/Z/04/Z. We thank Francesca Spiga for supplying experimental data. Useful discussions with Mohit Adhikari and Oscar Benjamin are also gratefully acknowledged.

References

Belchetz, P. E., Plant, T. M., Nakai, Y., Keogh, E. J. & Knobil, E. 1978 Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. *Science* **202**, 631-633.

Chrousos, G. P. 1995 The hypothalamic-pituitary-adrenal axis and immunemediated inflammation. *N. Engl. J. Med.* **332**, 1351-1362.

Dallman, M. F., Engeland, W. C., Rose, J. C., Wilkinson, C. W., Shinsako, J. & Siedenburg, F. 1978 Nycthemeral rhythm in adrenal responsiveness to ACTH. *Am. J. Physiol.* **235**, R210-R218.

Dallman, M. F., Akana, S. F., Cascio, C. S., Darlington, D. N., Jacobson, L. & Levin, N. 1987 Regulation of ACTH secretion: variations on a theme of B. *Recent Prog. Horm. Res.* **43**, 113-173.

Darmon, M., Brachet, P. & Da Silva, L. H. 1975 Chemotactic signals induce cell differentiation in *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3163-3166.

de Kloet, E. R., Joëls, M. & Holsboer, F. 2005 Stress and the brain: from adaptation to disease. *Nat. Rev. Neurosci.* **6**, 463-475.

Drouin, J., Sun, Y. L., Tremblay, S., Lavender, P., Schmidt, T. J., de Léan, A. & Nemer, M. 1992 Homodimer formation is rate-limiting for high affinity DNA binding by glucocorticoid receptor. *Mol. Endocrinol.* **6**, 1299-1309.

Engelborghs, K., Luzyanina, T. and Samaey, G. 2001 DDE-BIFTOOL v. 2.00: a Matlab package for bifurcation analysis of delay differential equations. Technical Report TW-330, Department of Computer Science, K.U. Leuven, Leuven, Belgium.

Gammaitoni, L., Hänggi, P., Jung, P. & Marchsoni, F. 1998 Stochastic resonance. *Rev. Mod. Phys.* **70**, 223-288.

Goldbeter, A. 1996 *Biochemical oscillations and cellular rhythms: the molecular basis of periodic and chaotic behaviour*. Cambridge (United Kingdom): Cambridge University Press.

Gupta, S., Aslakson, E., Gurbaxani, B. M. & Vernon, S. D. 2007 Inclusion of the glucocorticoid receptor in a hypothalamic pituitary adrenal axis model reveals bistability. *Theor. Biol. Med. Model.* **4**, 8.

Holsboer, F. 2000 The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* **23**, 477-501.

Holsboer, F. 2001 Stress, hypercortisolism and corticosteroid receptors in depression: implications for therapy. *J. Affect. Disord.* **62**, 77-91.

Iranmanesh, A., Lizarralde, G., Johnson, M. L. & Veldhuis, J. D. 1989 Circadian, ultradian and episodic release of beta-endorphin in men and its temporal coupling with cortisol. *J. Clin. Endocrinol. Metab.* **68**, 1019-1026.

Ixart, G., Barbanel, G., Nouguier-Soulé, J. & Assenmacher, I. 1991 A quantitative study of the pulsatile parameters of CRH-41 secretion in unanesthetized free-moving rats. *Exp. Brain Res.* **87**, 153-158.

Jasper, M. S. & Engeland, W. C. 1991 Synchronous ultradian rhythms in adrenocortical secretion detected by microdialysis in awake rats. *Am. J. Physiol.* **261**, R1257-R1268.

Jones, M. T., Hillhouse, E. W. & Burden, J. L. 1977 Dynamics and mechanics of corticosteroid feedback at the hypothalamus and anterior pituitary gland. *J. Endocrinol.* **73**, 405-417.

Krauskopf, B., Osinga, H. M. & Galán-Vioque, J. (eds.) 2007 *Numerical Continuation Methods for Dynamical Systems: Path following and boundary value problems*. Springer.

Kuznetsov, Y. A. 1995 *Elements of Applied Bifurcation Theory*. Springer.

Lightman, S. L., Wiles, C. C., Atkinson, H. C., Henley, D. E., Russell, G. M., Leendertz, J. A., McKenna, M. A., Spiga, F., Wood, S. A. & Conway-Campbell, B. L. 2008 The significance of glucocorticoid pulsatility. *Eur. J. Pharmacol.* **583**, 255-262.

Lui, J. H., Kazer, R. R. & Rasmussen, D. D. 1987 Characterization of the twenty-four hour secretion patterns of adrenocorticotropin and cortisol in normal women and patients with Cushing's disease. *J. Clin. Endocrinol. Metab.* **64**, 1027-1035.

McEwen, B. S. 2007 Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* **87**, 873-904.

Mershon, J. L., Sehlhorst, C. S., Rebar, R. W. & Liu, J. H. 1992 Evidence of a corticotrophin-releasing hormone pulse generator in the macaque hypothalamus. *Endocrinology* **130**, 2991-2996.

Nemeroff, C. B. 1996 The corticotropin-releasing factor (CRF) hypothesis of depression: new findings and new directions. *Mol. Psychiatry* **1**, 336-342.

Papaikonomou, E. 1977 Rat adrenocortical dynamics. *J. Physiol.* **265**, 119-131.

Papavasiliou, S. S., Zmeili, S., Khoury, S., Landefeld, T. D., Chin, W. W. & Marshall, J. C. 1986 Gonadotropin-releasing hormone differentially regulates expression of the genes for luteinizing hormone alpha and beta subunits in male rats. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4026-4029.

Pariante, C. M. & Miller, A. H. 2001 Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol. Psychiatry* **49**, 391-404.

Pariante, C. M. 2003 Depression, stress and the adrenal axis. *J. Neuroendocrinol.* **15**, 811-812.

Pariante, C. M. 2004 Glucocorticoid receptor function in vitro in patients with major depression. *Stress* **7**, 209-219.

Pepin, M. C., Pothier, F. & Barden, N. 1992 Antidepressant drug action in a transgenic mouse model of the endocrine changes seen in depression. *Mol. Pharmacol.* **42**, 991-995.

Reppert, S. M. & Weaver, D. R. 2002 Coordination of circadian timing in mammals. *Nature* **418**, 935-941.

Spiga, F., Harrison, L. R., Wood, S. A., Atkinson, H. C., MacSweeney, C. P., Thomson, F., Craighead, M., Grassie, M. & Lightman, S. L. 2007 Effect of the glucocorticoid receptor antagonist Org 34850 on basal and stress-induced corticosterone secretion. *J. Neuroendocrinol.* **19**, 891-900.

Stavreva, D. A., Wiench, M., John, S., Conway-Campbell, B. L., McKenna, M. A., Pooley, J. R., Johnson, T. A., Voss, T. C., Lightman, S. L. & Hager, G. L. 2009 Ultradian hormone

stimulation induces glucocorticoid receptor-mediated pulses of gene transcription. *Nat. Cell Biol.* **11**, 1093-1102.

Stricker, J., Cookson, S., Bennett, M. R., Mather, W. H., Tsimring, L. S. & Hasty, J. 2008 A fast, robust and tunable synthetic gene oscillator. *Nature* **456**, 516-519.

Tigges, M., Marquez-Lago, T. T., Stelling, J. & Fussenegger, M. 2009 A tunable synthetic mammalian oscillator. *Nature* **457**, 309-312.

Tsai, T. Y., Choi, Y. S., Ma, W., Pomerening, J. R., Tang, C. & Ferrell Jr, J. E. 2008 Robust, tunable biological oscillations from interlinked positive and negative feedback loops. *Science* **321**, 126-129.

Ulrich-Lai, Y. M. & Herman, J. P. 2009 Neural regulation of endocrine and autonomic stress responses. *Nat. Rev. Neurosci.* **10**, 397-409.

Veldhuis, J. B., Iranmanesh, A., Lizarralde, G. & Johnson, M. L. 1989 Amplitude modulation of a burst-like mode of cortisol secretion subserves the circadian glucocorticoid rhythm. *Am. J. Physiol.* **257**, E6–E14.

Veldhuis, J. B., Iranmanesh, A., Johnson, M. L. & Lizarralde, G. 1990 Amplitude, but not frequency, modulation of adrenocorticotropin secretory bursts gives rise to the nyctohemeral rhythm of corticotropic axis in man. *J. Clin. Endocrinol. Metab.* **71**, 452-463.

Waxman, D. J., Ram, P. A., Park, S. H. & Choi, H. K. 1995 Intermittent plasma growth hormone triggers tyrosine phosphorylation and nuclear translocation of a liver-expressed, Stat 5-related DNA binding protein. Proposed role as an intracellular regulator of male-specific liver gene transcription. *J. Biol. Chem.* **270**, 13262-13270.

Webster, M. J., Knable, M. B., OGrady, J., Orthmann, J. & Weickert, C. S. 2002 Regional specificity of brain glucocorticoid receptor mRNA alterations in subjects with schizophrenia and mood disorders. *Mol. Psychiatry* **7**, 985-994.

Weitzman, E. D., Fukushima, D., Nogeire, C., Roffwarg, H., Gallagher, T. F. & Hellman, L. 1971 Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *J. Clin. Endocrinol. Metab.* **33**, 14-22.

Wiesenfeld, K. & Moss, F. 1995 Stochastic resonance and the benefits of noise: from ice ages to crayfish and SQUIDs. *Nature* **373**, 33-36.

Wildt, L., Häusler, A., Marshall, G., Hutchison, J. S., Plant, T. M., Belchetz, P. E. & Knobil, E. 1981 Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. *Endocrinology* **109**, 376-385.

Windle, R. J., Wood, S. A., Shanks, N., Lightman, S. L. & Ingram, C. D. 1998 Ultradian rhythm of basal corticosterone release in the female rat: dynamic interaction with the response to acute stress. *Endocrinology* **139**, 443-450.

Windle, R. J., Wood, S. A., Lightman, S. L. & Ingram, C. D. 1998 The pulsatile characteristics of hypothalamo-pituitary-adrenal activity in female Lewis and Fischer 344 rats and its relationship to differential stress responses. *Endocrinology* **139**, 4044-4052.

Young, E. A., Abelson, J. & Lightman, S. L. 2004 Cortisol pulsatility and its role in stress regulation and health. *Front. Neuroendocrinol.* **25**, 69-76.

Young, E. A., Ribeiro, S. C. & Ye, W. 2007 Sex differences in ACTH pulsatility following

metyrapone blockade in patients with major depression. *Psychoneuroendocrinology* **32**, 503-507.

Figure captions

Figure 1. Regulation of HPA axis activity. The hypothalamic paraventricular nucleus (PVN) receives circadian inputs from the suprachiasmatic nucleus (SCN) and homeostatic/stress inputs from the brainstem and limbic areas. The PVN projects to the median eminence where it releases CRH into the portal circulation. This passes to corticotrophs in the anterior pituitary which release ACTH from pre-formed granules into the venous circulation. This ACTH reaches the adrenal cortex where it activates the synthesis and secretion of CORTisol (in man) or CORTicosterone (in the rodent). CORT in turn feeds back to inhibit release of ACTH from the anterior pituitary, and to a lesser extent, CRH from the hypothalamus.

Figure 2. Experimental data demonstrating the ultradian glucocorticoid rhythm underlying the classic circadian profile. Levels of blood corticosterone were recorded over a 24-hour period in two individual male Sprague-Dawley rats. Blood samples were collected every 10 min using an automated blood sampling system. Grey bars indicate the dark phase (19.15 - 05.15 hr). Data from Spiga *et al.* (2007).

Figure 3. Response of the pituitary-adrenal system to constant CRH drive. Units of all hormone levels are arbitrary. (*a*) Different combinations of constant CRH drive and delay can lead to two qualitatively different responses. On one side of the transition curve, the pituitary-adrenal system responds with constant levels in ACTH and CORT. On the other side of the transition curve, the pituitary-adrenal system responds with pulsatile fluctuations in the levels of ACTH and CORT, despite the fact that the CRH drive is constant. In the region of pulsatile response, the frequency of the pulses is indicated by the colour-bar. (*b*,*c*,*d*) Model predictions for ACTH (blue) and

CORT (black). Each time series was computed with the same delay (10 min) but different levels of constant CRH drive, as indicated by the three points in panel (*a*).

Figure 4. Frequency of CORT pulses inside the pulsatile region. Frequency of ultradian CORT rhythm computed for different values of the adrenal delay T_{lag} (units minutes) and different levels of CRH drive (arbitrary units). For all four values of the delay we observe ultradian pulses at physiological frequencies. See also the colour-bar in figure 3*a*.

Figure 5. Response of the pituitary-adrenal system to circadian and ultradian patterns of CRH drive. Units of all hormone levels are arbitrary. (*a*) Different combinations of constant CRH drive and delay can lead to two qualitatively different responses. On one side of the transition curve, the pituitary-adrenal system responds with constant levels in ACTH and CORT. On the other side of the transition curve, the pituitary-adrenal system responds with pulsatile fluctuations in the levels of ACTH and CORT, despite the fact that the CRH drive is constant. In the region of pulsatile response, the frequency of the pulses is indicated by the colour-bar. (*b*) Experimental data demonstrating an increase in pulse amplitude during the circadian peak. Data from Spiga *et al.* (2007). (*c*) Model prediction for a noisy circadian CRH drive close to (but below) the pulsatile region, as indicated by the corresponding arrow in panel (*a*). Response demonstrates noise-induced coherent oscillations (NICO) during the peak of the circadian CRH drive. Computed with a delay of 9.4 min. (*d*) Model prediction for a circadian CRH drive in the pulsatile region, as indicated by the corresponding arrow in panel (*a*). Response demonstrates increased pulse amplitude during the peak of the circadian CRH drive. Computed with a delay of 15 min. (*e*) Model prediction for ultradian pulses of CRH drive in the pulsatile region, as indicated by the corresponding arrow in panel (*a*). Response demonstrates a frequency in CORT governed by the

pituitary-adrenal system and not by the frequency of the CRH forcing. Computed with a delay of 12 min. (*f*) Model prediction for ultradian pulses of CRH drive in the region of constant response, as indicated by the corresponding arrow in panel (*a*). Response demonstrates a frequency in CORT that is governed by the frequency of the CRH forcing. Computed with a delay of 12 min.

Figure 6. Effect of subchronic treatment with a GR antagonist on the 24 hr corticosterone profile. (*a*) Data points represent mean levels of blood corticosterone measured from individual male Sprague-Dawley rats injected twice a day for five days with either the GR antagonist Org 34850 (10 mg/kg, s.c., n = 7, grey dots) or VEH (5% mulgofen in 0.9% saline, 1 ml/kg, s.c., n = 7, black dots). Blood samples were recorded over a 24-hour period and collected every 10 min using an automated blood sampling system. Also shown are curves numerically fitted to the two data sets, demonstrating an increase in amplitude during the circadian peak under the influence of Org 34850. Grey bar represents the dark phase (19.15-05.15 hr). Data from Spiga *et al.* (2007). (*b*) Model predictions using a circadian CRH drive demonstrate that the infusion of a GR antagonist (such as Org 34850) increases the amplitude of the ultradian glucocorticoid rhythm during the peak of the circadian CRH drive together with, a minor increase in ultradian frequency. Computed with a delay of 15 min.

Figures

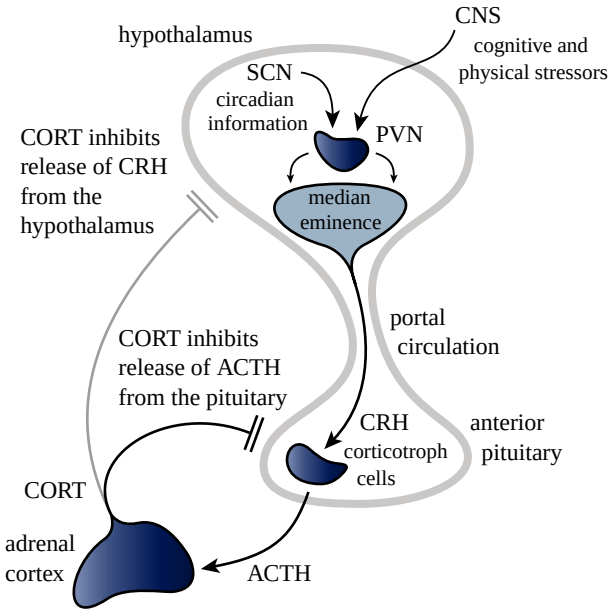

Figure 1:

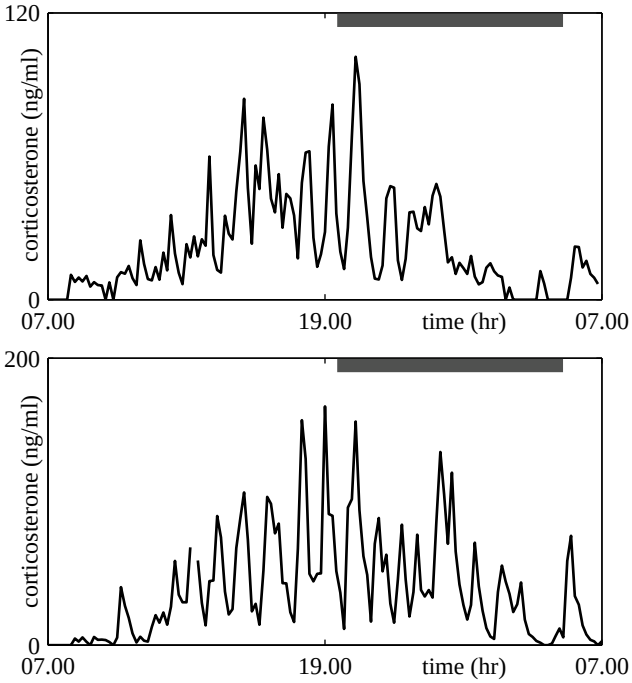
120
corticosterone (ng/ml)
0
07.00
19.00
time (hr)
07.00
200
corticosterone (ng/ml)
0
07.00
19.00
time (hr)
07.00

Figure 2:

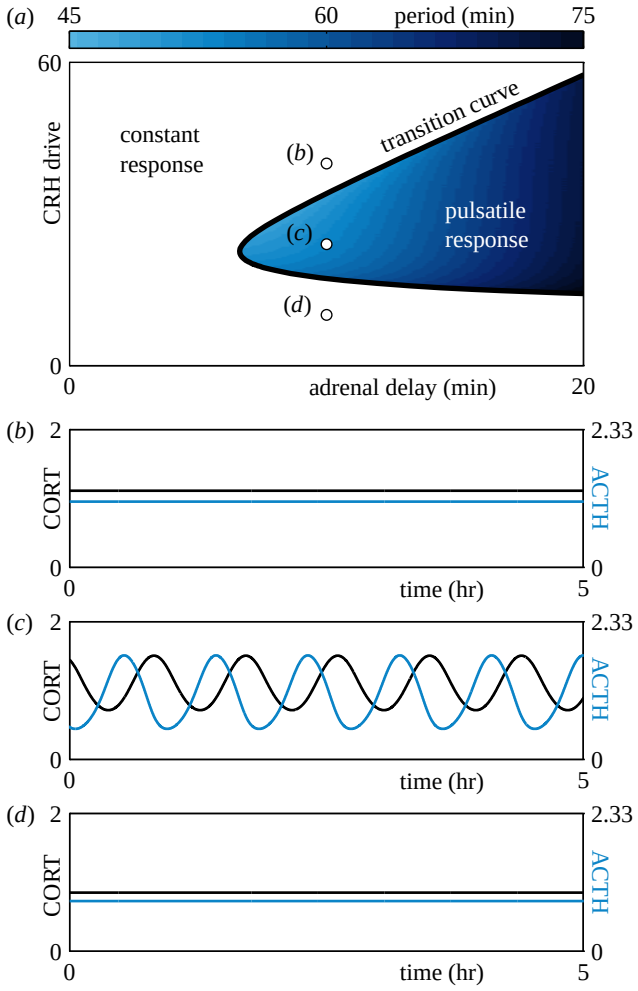
(a)
45
60
period (min)
75
60
CRH drive
constant
response
(b)
transition curve
pulsatile
response
(c)
(d)
0
0
adrenal delay (min)
20
(b)
2
2.33
CORT
ACTH
0
0
0
time (hr)
5
(c)
2
2.33
CORT
ACTH
0
0
0
time (hr)
5
(d)
2
2.33
CORT
ACTH
0
0
0
time (hr)
5

Figure 3:

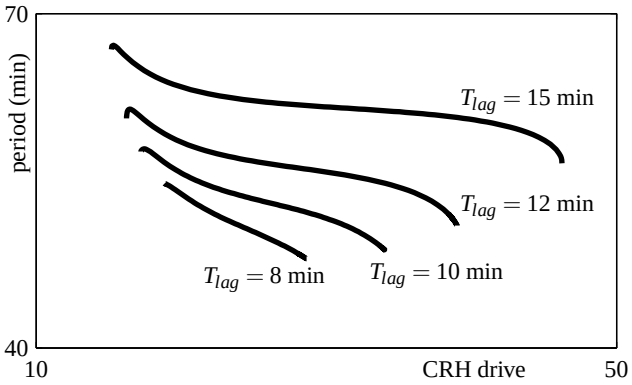

Figure 4:

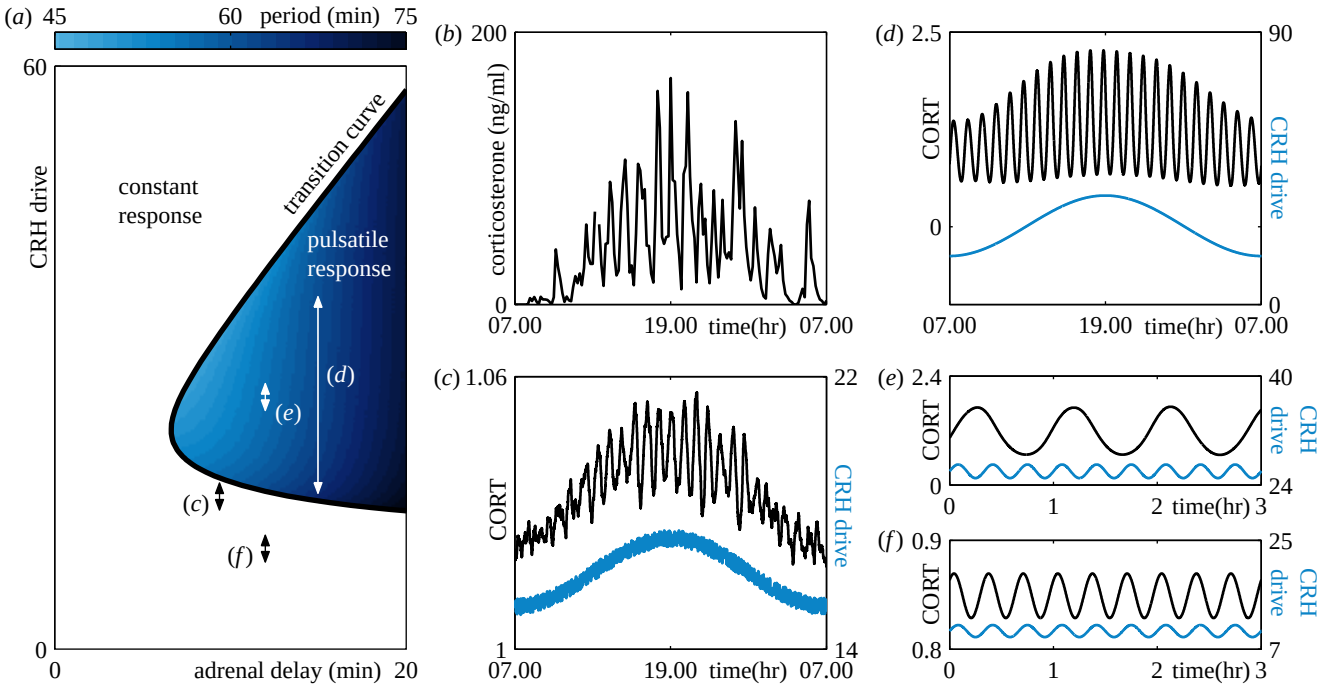

Figure 5:

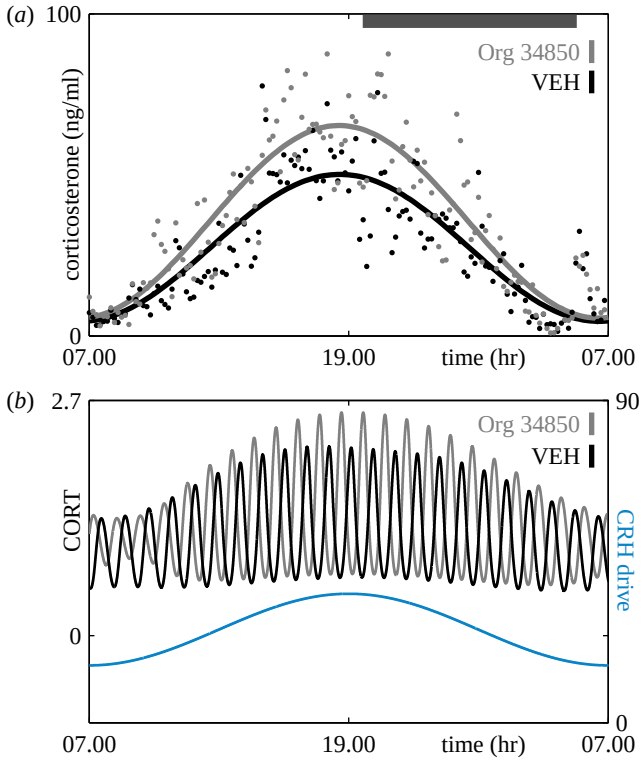
(a) 100
Org 34850
VEH
corticosterone (ng/ml)
0
07.00
19.00
time (hr)
07.00
(b) 2.7
90
Org 34850
VEH
CORT
CRH drive
0
0
07.00
19.00
time (hr)
07.00

Figure 6: